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(54) Title: USE OF TRANSFORMING GROWTH FACTOR β AND GROWTH FACTORS IN THE TREATMENT AND PREVENTION OF DISEASES OF THE INTESTINAL MUCOSA

(57) Abstract: The present invention relates to the use of transforming growth factor β(TGF-β) and anabolic growth factors (AGF) in the treatment and/or prevention of malfunction or disease of the intestinal mucosa. More in particular the invention relates to the treatment and/or prevention of damage of the intestinal mucosa as a result of chemotherapy of radiotherapy or of inflammatory bowel diseases with a product comprising: a) a first pharmaceutical composition comprising TGF-β in the substantial absence of insulin-like growth factor-1(IGF-1); b) a second pharmaceutical composition comprising AGF in the substantial absence of TGF-β;

USE OF TGF BETA AND GROWTH FACTORS IN THE TREATMENT AND PREVENTION OF DISEASES OF THE INTESTINAL MUCOSA

The present invention relates to the use of a composition containing transforming growth factor β (TGF-β) and a composition containing anabolic growth factors (AGF), in particular insulin-like growth factor 1 (IGF-1) for the prevention or treatment of malfunction or disease of the intestinal mucosa. The invention further relates to a composition containing TGF-β and specific fibres and/or immunoglobulines and/or calcium which can also be used for such a treatment, in particular in combination with IGF-1. The composition containing TGF-β is administered during a period in which it is desired to inhibit cell proliferation and stimulate cell differentiation. The composition containing IGF-1 is administered to restore intestinal epithelial cells.

TGF-β is a multifunctional protein found in all mammalian tissues. Currently, five forms of TGF-β are known, β1 to β5. It has been implicated in the development, differentiation and growth of tissue and the control of immune system function and carcinogenesis. TGF-β can be isolated from natural sources (e.g. blood platelets), mammalian milk or colostrum or can be produced by recombinant cells.

IGF-1 is a small protein (molecular weight about 7800) which plays an important role in bone metabolism. It has been shown to stimulate growth of cells in culture. Animal growth is also stimulated in pituitary deficient, normal and catabolic states. Kidney function is also improved. It can be produced using recombinant DNA technology, solid phase peptide synthesis, by isolating it from blood serum or from human or bovine milk.

TGF-β and its uses are for instance described in EP 852913. This document relates to an enteral food preparation which contains casein rich in TGF-β2, a lipid source such as medium chain or long chain triglycerides or polyunsaturated fatty acids and a carbohydrate source, i.e. maltodextrin, corn starch or sucrose. This composition is used in the treatment or prophylaxis of inflammatory conditions of the gastrointestinal tract, such as Crohn's disease.

EP 462,398 describes the combination of TGF-β1 and a polyunsaturated fatty acid (PUFA) such as linoleic acid, alpha-linolenic acid, gamma-linolenic acid, arachidonic acid, dihomo-

gamma-linolenic acid, eicosapentaenoic acid and/or docosahexanoic acid and/or a derivative thereof for treatment of neoplastic diseases.

WO 96/34614 describes a method for preventing and/or treating damage to the lining of the alimentary tract resulting from chemotherapy and/or radiation, wherein a milk product extract including a mixture of cell growth factors is administered to a patient. The milk product extract, preferably a cheese whey extract, may contain lactoferrin and lactoperoxidase and it can be supplemented with growth factors such as IGF-1, IGF-2, TGF-β, TGF-α, EGF, PDGF, FGF or KGF. This document does not mention which of the substances mentioned should be present in order to achieve the desired preventing or curing effect.

In an article of S.T. Sonis et al, Cancer Res. 54:1135-1138 (1994); "Prevention of chemotherapy induced ulcerative mucositis by transforming growth factor β3" it is described that TGF-β3 administration reduced proliferation of oral epithelium in vitro and in vivo. Topical application of TGF-β3 to the oral mucosa of the Syrian golden hamster prior to chemotherapy significantly reduced the incidence, severity and duration of oral mucositis, reduced chemotherapy associated weight loss and increased survival. Prevention of mucositis according to this document is based on limiting the rate of basal epithelial cell proliferation by prior administration of a negative growth regulator.

It was found according to the invention that for optimum protection of the intestinal mucosa against the damaging effect of chemotherapy and radiotherapy TGF-β should be administered without the presence of IGF-1, in particular any anabolic growth factor, during the chemotherapy or radiotherapy. It was furthermore found that after the chemotherapy or radiotherapy, damaging effects that may have occurred during this therapy can be treated by administering anabolic growth factors, in particular IGF-1 in the substantial absence of TGF-β. According to the invention it was also found that the same sequential administration of TGF-β and IGF-1 can be beneficial in case of inflammatory bowel diseases (IBD), such as Crohn's disease.

The present invention therefore provides the use of TGF-β and AGF in the preparation of a product for use in the treatment and/or prevention of malfunction or disease of the intestinal mucosa; the product comprising:

a) a first pharmaceutical composition comprising TGF-β in the substantial absence of IGF-1;
b) a second pharmaceutical composition comprising AGF in the substantial absence of TGF-β;

wherein the first and second composition are administered sequentially.

Preferably, the weight ratio TGF-β/IGF-1 in the first pharmaceutical composition is at least 100.

It has been found that when a mixture of growth factors is used, for instance when a milk product extract is used, the beneficial effect of TGF-β is reduced by the presence of anabolic growth factors. Therefore, it is preferred to administer TGF-β in the substantial absence of such growth factors.

According to a preferred embodiment of the invention the first pharmaceutical composition comprises TGF-β in substantial absence of AGF. With AGF any anabolic growth factor is meant, i.e any growth factor that would promote cell growth. Examples thereof are: IGF-1, insulin-like growth factor 2 (IGF-2), growth hormone, epidermal growth factor (EGF), transforming growth factor α (TGF-α), mammalian milk growth factor (MMGF = Beta-cellulin) and fibroblast growth factor (FGF). EGF is for instance described in EP 0546 068, MMGF in WO 99/24470. The ratio TGF-β/AGF is preferably at least 50.

Preferably, the AGF in the second pharmaceutical composition is IGF-1. This means that preferably at least IGF-1 is present in the second composition.

According to the present invention when growth factors are mentioned, these include also the active peptide analogues of these growth factors. With peptide analogue is meant any peptide having substantially the same activity as the growth factor, particularly any peptide analogue which is 90% or more homologous with the growth factor.

"Pharmaceutical composition" according to the present invention is meant to include any conventional pharmaceutical preparation such as a capsule, a tablet etc., as well as dietetic preparations such as feed supplements or total feeds.

The sequential administration of the first pharmaceutical composition containing TGF-β and the second pharmaceutical composition containing AGF, preferably at least IGF-1, according to the invention is particular suitable for intestinal disorders in which two phases can be distinguished. The first phase is a phase in which it is desired to inhibit the metabolism. During the second phase, which follows the first phase, the intestinal epithelial cells need to be restored. The composition containing TGF-β is administered during the first phase, the composition containing anabolic growth factors, in particular IGF-1 during the second phase.

More in particular, the sequential administration of TGF-β and IGF-1 is used for the prevention and/or treatment of damage of the intestinal mucosa as a result of chemotherapy and/or radiotherapy. By "damage" is meant any alteration in normal structure or function. Such damage includes mucositis, at least partial loss of mucosal crypt area and/or mucosal villus length, or an increase in bacterial translocation across the alimentary tract.

Chemotherapy and/or radiotherapy are effective at destroying tumours because they target fast-growing tissues. While tumour cells are selectively targeted by anticancer treatments the fast growing tissues of the host are also susceptible, particularly the immune cells of the body and the lining of the alimentary tract. This can result in damage to the linings of the mouth and oesophagus (mucositis, also referred to as stomatitis) and damage to the intestinal lining, commonly in the small bowel and less frequently in the large bowel, leading to severe diarrhoea and pain.

It was found according to the invention that for optimum protection of the intestinal mucosa against the damaging effect of chemotherapy and radiotherapy a first composition containing TGF-β should be administered without the presence of IGF-I, preferably any anabolic growth factor, during the chemotherapy or radiotherapy, in particular during at least the period starting at the latest the first day of said chemotherapy or radiotherapy treatment and ending at the latest the last day of treatment. It was furthermore found that after the chemotherapy or radiotherapy, damaging effects that may have occurred during these therapies can be treated by administering a second composition containing AGF, in particular IGF-1, in the substantial absence of TGF-β.

According to a further embodiment of the invention the sequential administration of TGF-β and IGF-1 is used in the prevention and/or treatment of inflammatory conditions of the intestine, in particular inflammatory bowel diseases (IBD), such as Crohn's disease.

As the TGF-β to be used according to the present invention, every presently available TGF β- can be used e.g. TGF-β1 to TGF-β5. The TGF-β can be of both human and animal origin. Examples thereof are TGF-β which is produced by recombinant cells, TGF-β extracted from blood platelets and TGF-β extracted from milk or whey. Preferably a TGF-β extracted from a mammalian milk product, in particular bovine milk or whey is used because of the reluctance against products obtained by recombinant techniques, cost effectiveness and the presence of other beneficial components in milk or whey extract, such as immunoglobulins. A process for extracting such a TGF-β is described in a copending application of the applicants.

A TGF-β obtained from bovine whey or milk will in general contain more than 100, preferably more than 700 μg TGF-β per g protein. Such an extract will for instance contain 750 μg TGF-β/g protein. The IGF-1 content in this extract will be less than 4, preferably less than 1 μg/g protein.

Preferably the TGF-β is present in the composition in such an amount that 50 ng to 150 μg per day is administered. In case of a liquid product, this will contain TGF-β in a concentration of 0.5 μg -1.5 mg TGF-β per litre. The patient will be administered about 100 ml per day of such a liquid product.

It is preferred that the first pharmaceutical composition according to the invention also contains fibres which upon fermentation form more than 15 g of butyrate per 100 g of short chain fatty acids, preferably more than 20 g of butyrate per 100 g of short chain fatty acids. This characteristic means that the composition should contain fibres that release a relative large amount of butyrate when they are fermented in the intestine (colon). The amount of butyrate can be determined by the method described in Journal of Clinical Nutrition, 1991, no. 53, p. 1418-1424.

Certain disorders of gut function, for instance resulting from chemotherapy can influence the intestinal flora, which causes a temporary decrease of the fermentation to butyrate, in par-

ticular in those cases that the patient is also given a large amount of antibiotics. It is therefore important to administer fibres to the patient which stimulate the synthesis of butyrate by bacteria whereby more butyrate is released into the intestine. Butyrate is a preferential energy substrate in certain intestinal cells, and it also inhibits proliferation and increases differentiation of these cells.

If butyrate is administered as its free salt, undesirable off-flavours can occur. Further only part of the butyrate would reach the colon. A sustained release preparation could overcome this problem, however, these preparations are relatively expensive.

Therefore, according to the present invention it is proposed to administer specific fibres which upon fermentation result in butyrate. Such fibres are: resistant starch, oats bran, in particular the arabinoxylan rich fraction that is poor in β-glucan, some soy fibre extracts and wheat bran. Preferably, wheat bran is used. The amount of fibre is such that a daily ratio of 1 to 30 g, preferably 3 to 10 g, is obtained. In a liquid preparation the concentration is thus 10 to 300 g/l.

The TGF-β composition according to the invention preferably contains immunoglobulins, more in particular in combination with the above mentioned fibres. Their main function is to interact with harmful micro-organisms such as bacteria. This prevents the micro-organism from entering the blood circulation system. This situation in particular occurs when the intestinal mucosa of the patient has been damaged as a result of treatment with chemotherapy.

The immunoglobulins can be isolated from milk of mammals which have been hyperimmunised against certain pathogens or they can be isolated from normal bovine whey or milk. With the process described in the above mentioned patent application, using normal cow's milk as a starting material, a preparation is obtained rich in IgG and IgA. 30 to 50 % of the protein fraction consists of immunoglobulins of the type IgG and IgA. The concentration immunoglobulins in the preparation will, in case of a liquid preparation of 100 ml, be 0.1 to 1500 mg/l.

According to a further preferred embodiment the TGF-β composition contains calcium, preferably in combination with the above mentioned fibres, more preferably in combination with said fibres and immunoglobulins. The calcium can be in the form of finely dispersed

calcium phosphate, calcium carbonate, calcium citrate or a calcium concentrate from bovine milk. The addition of calcium reduces the risk of infection. Calcium lowers the proliferation rate of the epithelial cells. The amount of calcium is more than 50 mg/100 ml, preferably more than 100 mg/100 ml, for instances 120 mg/100 ml, based on a liquid composition.

It has been found that when a product containing TGF-β, butyrate producing fibres and high levels of calcium salts is administered a synergistic effect occurs resulting in a composition that is effective in preventing damage to epithelial gut cells during chemotherapy and radiotherapy and in the treatment of inflammatory bowel diseases.

Preferably, the first pharmaceutical composition containing TGF-β according to the invention also contains one or more of the following ingredients: proteins, fat, minerals, trace elements, vitamins, fatty acids and lactoferrin.

Preferably, proteins are present in an amount of 3 to 10 % protein equivalents, this includes intact protein, peptides and amino acids. The amount of fat is preferably 2 to 10 %, based on the total weight of the preparation. The amount of minerals, trace elements and vitamins is according to the daily recommended dosage.

Preferred vitamins are vitamin A, C and E. Vitamin A and provitamin A are required. Their concentration is preferably more than 130 μgRE/100 ml, in particular more than 300 μg/100 ml. Suitably part of the vitamin A is administered as retinoic acid or a metabolic equivalent thereof. Vitamin C and tocopherols are administered because of their role in the antioxidant cascade. During radiotherapy but also with initial inflammatory reactions they can protect the epithelial cells. The concentration vitamin C or an equivalent thereof is more than 40 mg/100 ml, preferably more than 60 mg/100 ml. The concentration of tocopherols is more than 5 mg, preferably more than 15 mg/100 ml.

The fats should provide sufficient fatty acids. Preferably stearidonic acid (STA) is added. Suitable fatty acids and the amounts and ratios in which they are used are described in PCT/EP98/08409, i.e. the fatty acids gamma-linolenic acid, stearidonic acid and eicosapentaenoic acid together constitute 10 to 500 mg/g of the total amount of fatty acids and

gamma-linolenic acid and eicosapentaenoic constitute 20 to 50 wt.% and stearidonic acid forms 15 to 50 wt.% of these three fatty acids.

Lactoferrin can be present because it has anti-bacterial activity against a number of pathogens. This substance can also have a modulating action with initial inflammatory reactions, which are delayed. It is desired to have a daily doses of 0.1 to 3 g of lactoferrin.

It is preferred that the composition contains less than 11 %, preferably less than 6 % digestible carbohydrates. A higher percentage of these substances would affect the taste of the composition. Generally about 4.5 g/100 ml are used. As a source of digestible carbohydrates sucrose, but also slowly digestible carbohydrates can be used.

In the second composition, the dosage of IGF-1 is preferably 0.1 to 100 μg IGF-1 per kg body weight per day. In a liquid product this concentration is 7 μg to 7 mg IGF-1 per 100 ml. The second composition preferably contains substantially no TGF-β. The ratio IGF-1/TGF-β is at least 50, preferably at least 100.

The second composition can further contain immunoglobulins. In view of the severity of the mucositis which has developed, it is important to prevent and/or treat translocation of harmful substances, for instance micro-organisms. Preferably doses of 0.03 mg to 5 mg immunoglobulins per day are administered. If the IGF-1 is obtained from bovine milk, generally a preparation will be obtained containing 10 to 1000 mg Ig per 100 μg IGF-1.

Beside immunoglobulins, fibres can be present. As this composition is administered during a phase wherein the intestinal flora of the patient is extremely disrupted, a mixture of fibres is preferably administered. Preferably theses fibres are soluble non-starch polysaccharides, such as gum arabic or pectin, insoluble non-starch polysaccharides, such as cellulose and hemicellulose and oligosaccharides and/or resistant starch and/or lignin. An example of such a mixture is described in EP 0756828, which is incorporated by reference.

The second composition can further contain one or more of lactoferrin, glutamine and anti-oxidants. Glutamine must have a stable form. In a liquid product, glutamine rich peptides should be used or extracts from hydrolysates of glutamine rich proteins. The amounts of

lactoferrin and antioxidants are the same as in the first composition. Further the second composition may contain fat, protein and other microcomponents, such as minerals, vitamins and trace elements. Further, substances that support the total methionin metabolism can be present.

According to a further embodiment of the invention TGF-β in the substantial absence of insulin-like growth factor 1 IGF-1, in particular in the absence of AGF, and fibres which upon fermentation form more than 15 g of butyrate per 100g of short chain fatty acids and/or immunoglobulins are used for preparing a pharmaceutical composition for treatment and/or prevention of malfunction or disease of the intestinal mucosa, more in particular for treatment and/or prevention of damage of the intestinal mucosa as a result of chemotherapy or radiotherapy or for treatment and/or prevention of inflammatory bowel diseases.

The present invention also relates to a pharmaceutical composition containing TGF-β in the substantial absence of IGF-1, in particular in the absence of AGF, preferably in combination with fibres which upon fermentation form more than 15 g of butyrate per 100 g of short chain fatty acids and/or immunoglobulins.

In case of a liquid composition, such a composition contains per 100 ml

a) 50 ng to 150 μg TGF-β
b) 1 to 30 g fibres
c) 0.01 to 150 mg immunoglobulins
d) 0.03 to 1 g lactoferrin
e) > 50 mg calcium
f) fatty acids
g) > 130 μg RE vitamin A
h) > 40 mg vitamin C
i) > 5 mg tocopherols
j) 3 to 10% protein equivalents

For example, a suitable liquid TGF-β based formula contains per 100 ml:

a) 4 μg TGF-β
b) 5 g wheat bran

c) 2 mg immunoglobulins
d) 0.5 g lactoferrin
e) 80 mg calcium
f) 4 g fat blend containing 30 % MCT, 26 % palm oil, 16 % soy oil, 8 % borage oil, 11 % echium oil, 6.5 % fish oil and 2.5 % egg lipids
g) 300 µg vitamin A
h) 70 mg vitamin C
i) 15 mg α-tocopherol
j) 4 g casein
k) 5 g maltodextrin

Of this formula, 250 ml per day is administered.

The invention also relates to a pharmaceutical composition containing AGF, preferably IGF-1, in the substantial absence of TGF-β and fibres selected from soluble non-starch polysaccharides, such as gum arabic or pectin, insoluble non-starch polysaccharides, such as cellulose and hemicellulose and oligosaccharides and/or resistant starch and/or lignin. The composition preferably further contains at least one member of the group comprising lactoferrin, glutamine and antioxidants.

In case of a liquid composition, such a composition contains per 100 ml:

a) 7 µg to 7 mg IGF-1
b) 1 to 30 g fibres
c) 5 to 300 mg immunoglobulins
d) 0.3 to 3 g lactoferrin
e) 0.5 to 10 g glutamine
f) > 130 µg RE vitamin A
g) > 40 mg vitamin C
h) > 5 mg tocopherols

For example, a suitable liquid IGF-1 based formula contains per 100 ml:

a) 100 µg IGF-1
b) 5 g fibre mix: 1g wheat bran, 3 g inulin, 1 g oats bran
c) 200 mg immunoglobulins

d) 0.5 g bovine lactoferrin
e) 5 g alanylglutamine
f) 300 µg vitamin A
g) 70 mg vitamin C
h) 15 mg α-tocopherol

Of this formula, 250 ml per day is administered.

The compositions according to the invention can have the form of any oral preparation, for instance capsules, sachets or tablets each containing a predetermined amount of the active ingredient; powders or granules; solutions or suspensions in an aqueous or non-aqueous liquid. Preferred dosage forms are food supplements or total feeds or powders which upon reconstitution with a liquid such as water give a total feed or food supplement. The present invention also relates to tube feeds containing these ingredients.

The present invention also relates to products consisting of a combination of the first composition and the second composition for sequential administration for preventing and/or treating damage of the intestinal mucosa as a result of chemotherapy or radiotherapy or for preventing and/or treating inflammatory conditions of the intestine, in particular Crohn's disease.

Claims

1. Use of transforming growth factor β (TGF-β) and anabolic growth factors (AGF) in the preparation of a product for use in the treatment and/or prevention of malfunction or disease of the intestinal mucosa; the product comprising

a) a first pharmaceutical composition comprising TGF-β in the substantial absence of insulin-like growth factor-1 (IGF-1);

b) a second pharmaceutical composition comprising AGF in the substantial absence of TGF-β;

wherein the first and second composition are administered sequentially.

2. Use according to claim 1, wherein the first pharmaceutical composition comprises TGF-β in substantial absence of AGF.

3. Use according to claim 1 or 2, wherein the second pharmaceutical composition comprises at least IGF-1 as the AGF.

4. Use according to any of claims 1 to 3, for the preparation of a product for use in the treatment and/or prevention of damage of the intestinal mucosa as a result of chemotherapy or radiotherapy.

5. Use according to claim 4, wherein the first pharmaceutical composition is administered during the period starting at the latest the first day of said chemotherapy or radiotherapy treatment and ending at the latest at the effective end of said treatment.

6. Use according to any of claims 1 to 3, for the preparation of a product for use in the treatment and/or prevention of inflammatory bowel diseases.

7. Use according to any of claims 1 to 6, wherein the TGF-β is obtained by extraction from a mammalian milk product, preferably bovine milk or whey.

8. Use according to any of claims 1 to 7, wherein the first pharmaceutical composition further contains fibres which upon fermentation form more than 15 g of butyrate per 100 g of short chain fatty acids and/or immunoglobulins and/or calcium.

9. Use according to claim 8, wherein the first pharmaceutical composition contains fibres which upon fermentation form more than 15 g of butyrate per 100 g of short chain fatty acids as well as immunoglobulins and calcium.

10. Use according to claim 8 or 9, wherein the fibres are wheat bran fibres.

11. Use according to any of claims 1 to 10, wherein the first pharmaceutical composition further contains at least one member of the group of lactoferrin, fatty acids and antioxidants.

12. Use according to any of claims 1 to 11, wherein the second pharmaceutical composition further contains fibres selected from the group of soluble non-starch polysaccharides, insoluble non-starch polysaccharides, oligosaccharides, resistant starch and mixtures thereof.

13. Use according to any of claims 1 to 12, wherein the second pharmaceutical composition further contains at least one member of the group comprising lactoferrin, glutamine and antioxidants.

14. Product containing

a) transforming growth factor β (TGF-β) in substantial absence of insulin-like growth factor 1 (IGF-1) and

b) anabolic growth factors (AGF) in substantial absence of TGF-β;

as combination for sequential administration for treating and/or preventing malfunction or disease of the intestinal mucosa.

15. Pharmaceutical composition containing

a) transforming growth factor β (TGF-β) in the substantial absence of insulin-like growth factor 1 (IGF-1) and

b) fibres which upon fermentation form more than 15 g of butyrate per 100 g of short chain fatty acids and/or
c) immunoglobulins and/or
d) calcium.

16. Pharmaceutical composition according to claim 15, which comprises TGF-β in the substantial absence of anabolic growth factors (AGF).

17. Pharmaceutical composition according to claim 15 or 16, containing fibres as well as immunoglobulins and calcium.

18. Pharmaceutical composition according to any of claims 15 to 17, containing fibres in such an amount that 1 to 30 g of fibres per day are administered.

19. Pharmaceutical composition according to any of claims 15 to 18, wherein the fibres are wheat bran fibres.

20. Pharmaceutical composition according to any of claims 15 to 19, wherein the TGF-β is obtained by extraction from a mammalian milk product, preferably bovine milk or whey.

21. Pharmaceutical composition according to any of claims 15 to 20, containing TGF β in such an amount that 50 ng to 150 μg TGF-β per day is administered.

22. Pharmaceutical composition according to any of claims 15 to 21, further containing at least one member of lactoferrin, fatty acids and antioxidants.

23. Use of
a) transforming growth factor β (TGF-β) in the substantial absence of insulin-like growth factor 1 (IGF-1) and
b) fibres which upon fermentation form more than 15 g of butyrate per 100g of short chain fatty acids and/or
c) immunoglobulins and/or
d) calcium

for preparing a pharmaceutical composition for treatment and/or prevention of malfunction or disease of the intestinal mucosa.

24. Use according to claim 23, wherein TGF-β is applied in the substantial absence of anabolic growth factors (AGF).

25. Use according to claim 23 or 24, for preparing a pharmaceutical composition for treatment and/or prevention of damage of the intestinal mucosa as a result of chemotherapy or radiotherapy.

26. Use according to claim 23 or 24, for preparing a pharmaceutical composition for treatment and/or prevention of inflammatory bowel diseases.

27. Pharmaceutical composition containing

a) anabolic growth factors (AGF) in the substantial absence of transforming growth factor-β (TGF-β) and

b) fibres selected from the group of soluble non-starch polysaccharides, insoluble non-starch polysaccharides, oligosaccharides, resistant starch and mixtures thereof.

28. Pharmaceutical composition according to claim 27, containing at least insulin-like growth factor-1 (IGF-1) as the AGF.

29. Pharmaceutical composition according to claim 27 or 28, further containing at least one member of the group comprising lactoferrin, glutamine and antioxidants.

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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/NL 99/00620

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/18 A61K38/30 A23L1/30 //(A61K38/30,38:18), (A61K38/18,31:20,31:715,38:40,39:395),(A61K38/30,31:715,38:05, 38:40,39:395)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, FSTA, MEDLINE, CANCERLIT, AIDSLINE, LIFESCIENCES, CHEM ABS Data, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 824 297 A (IWATA K K ET AL) 20 October 1998 (1998-10-20) claims 1-12,27-29; examples 2-7B column 2, line 6 - line 10 column 6, line 25 -column 7, line 16	1-5,14
A	the whole document & US 5 817 625 A (HALEY JOHN DOUGLAS) 6 October 1998 (1998-10-06) column 2, line 23 - line 36	15-26

A	BECK P L & WALLACE J L: "Cytokines in inflammatory bowel disease." MEDIATORS OF INFLAMMATION, vol. 6, no. 2, April 1997 (1997-04), pages 95-103, XP000929627 table 2	6

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
2 October 2000	06. 10. 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Teyssier, B

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 99/00620

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

In claims 11, 13, 22 and 29 the designation "antioxydants" relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only two such compounds: Vitamins C and E (alpha-tocopherol). In the present case the claims so lack support and the application so lacks disclosure that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those two compounds which appear to be supported and disclosed and their immediate derivatives.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-14

   Use of TGF-beta and so called "anabolic" growth factors in the preparation of two separate compositions for sequential use in the treatment or prevention of diseases of the intestinal mucosa; product containing the two compositions.

2. Claims: 15-26

   Pharmaceutical composition comprising TGF-beta, butyrate-producing fibers, immunoglobulins, calcium.

3. Claims: 27-29

   Pharmaceutical composition comprising a so called "anabolic" growth factor and fibers.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 99/00620

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